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HEPATIC IODOTHYRONINE 5'-MONODEIODINASE ACTIVITY IN THE BROILER CHICKEN: EFFECT OF DIETARY FAT AND TRIIODOTHYRONINE (T_3) SUPPLEMENTATION

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ABSTRACT

The conversion of thyroxine (T_4) to the metabolically active thyroid hormone, triiodothyronine (T_3), is catalyzed by iodothyronine 5'-monodeiodinase (EC 3.8.1.4; 5'D). Indian River male broiler chickens growing from 7 to 28 d of age were used in a 3 x 2 factorial to determine the effect of dietary energy from fat and T_3 supplementation on hepatic 5'D activity and plasma concentration of T_4 and T_3. Chickens were fed diets (13.1 MJ/kg diet) containing 1.25 (LF), 2.5 (MF) and 5.00 (HF) MJ from fat/kg diet + 0 or 1 mg T_3/kg diet. Blood and liver samples were collected on d 28. Hepatic 5'D was affected by fat x T_3 interaction ($P<0.01$): with no added T_3, MF and HF increased 5'D 25 ($P<0.01$) and 16% ($P<0.05$) as compared to LF (1.5 nmoles I^- hr^{-1} · mg $protein^{-1}$); however no changes in 5'D were found when T_3 was added (1.42, 1.35 and 1.36 for LF, MF and HF, respectively). Diets with T_3 increased plasma T_3 (5.1 vs. 18.1 nmol/L, $P<0.001$) and decreased plasma T_4 (12.4 vs. 7.9 nmol/L, $P<0.001$). Dietary fat did not affect plasma T_3 and T_4. The data indicate that the hepatic generation of T_3 is stimulated by increased dietary fat intake. This effect of fat, however, is inhibited by dietary T_3 supplementation.
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KEY WORDS: Chickens, Thyroid, Dietary Fat, Enzymes, Deiodination, Metabolism

INTRODUCTION

Thyroid hormones regulate both the metabolism of chickens and, possibly, the flux of calories required to support metabolism. In addition, thyroid hormones may alter sensitivity of an organ to other regulatory hormones and tissue factors as well as directly influence metabolic rate of that organ (1). Although thyroxine (T_4) is the predominant thyroid hormone in circulation, it has little inherent biological activity (2). The more metabolically active thyroid hormone, triiodothyronine (T_3), is produced by the 5'-deiodination of thyroxine (T_4) catalyzed by iodothyronine 5'-monodeiodinase (5'D; EC 3.8.1.4; 5'D) within the thyroid gland and in extrathyroidal tissues (2,3). Because T_3 is a very potent regulator of energy and protein

metabolism(3), the extrathyroidal activity of 5'D is an important control point for regulating thyroid hormone levels in various physiological situations (4).

It has been shown that fasting and low calorie diets decrease, while refeeding, hyperphagia and high carbohydrate diets increase extrathyroidal 5'D activity in mammals (5,6). Increased hepatic 5'D activity was also reported in rats fed a high fat diet (7) and in sheep fed a palm oil (8) or a soy lecithin supplemented diet (9). It is known, at least in mammals, that hepatic 5'D activity (type I) is stimulated by increased concentrations of T_4 (4). This increase in enzyme activity could result from either an increase in enzyme protein or from a more favorable substrate concentration. The latter explanation is certainly feasible because plasma T_4 circulates at concentrations (0.01 - 0.1 x 10^{-6} M) far less than the apparent Km (1 x 10^{-6} M). While no comprehensive data are available for birds, decreased 5'D activities were reported in both hypothyroid mammals (4) and birds (10) . Recently, we have shown that feeding T_4 (11) or T_3 (12) to growing chickens decreased body weight gain, total lipid content and *in vitro* lipogenesis without directly affecting the activity of several lipogenic enzymes. These findings indicate that there is a close relationship between thyroid hormone, thyroid hormone metabolism, and fat metabolism and deposition.

The purpose of the present study was to investigate the interaction of dietary fat levels and dietary T_3 on hepatic activity of Type I 5'D and on plasma concentrations of T_3 and T_4 in growing broiler chickens.

MATERIALS AND METHODS

Animals, Diets, and Treatments Male Indian River broiler chickens were fed a diet containing 21% crude protein from 1 to 7 d of age. At 7 d of age they were randomly assigned to a 2 x 3 factorial arrangement of 6 dietary treatments (13.1 MJ/kg diet; 1.25 [LF], 2.5 [MF], and 5.0 [HF] MJ from fat/kg diet + 0 or 1 mg T_3 /kg diet) for days 7 to 28 d of the study. Fat content of each diet was derived by adding together the reported fat contents for soya-bean meal, maize meal and maize oil. The ingredients and composition of the diets are presented in Table 1. The chickens (9/treatment) were housed in an environmentally controlled room at 23 C with a 12-h light-dark cycle (0600-1800 light). On d 28, chickens were bled by cardiac venipuncture and killed by cervical dislocation. Blood plasma was stored at -20 C until assayed for T_4 and T_3 . Livers were immediately frozen in liquid nitrogen and stored at -80 C before being assayed for 5'D activity.

All chickens were held under a quarantine that was certified by the station veterinarian. Chickens were observed daily for healthiness. One authorized animal caretaker was assigned to maintain chickens over the course of the experiments. In addition, the research protocols were approved by the Beltsville Agricultural Research Center Animal Care Committee.

Hormone Determination Plasma T_3 and T_4 concentrations were determined using commercially available solid-phase RIA kits (ICN, Biomedicals Inc., Carson, CA) with standards prepared in chicken T_4- and T_3-free serum. Hormone-free serum was obtained by adding charcoal to serum, followed by a brief incubation and centrifugation Assays were validated for avian samples as described by Rosebrough *et al.* (11).

Iodothyronine 5'-Deiodinase Determination (type I) Outer-ring deiodinating activity (5'D) was determined by measuring the ^{125}I released from [^{125}I]-rT_3 (reverse-T_3) according to a modified method of Leonard and Rosenberg (13). Outer ring deiodination is the predominant form of deiodination in chicken livers and results in T_3 formation. This method avoids the complexity

of using an RIA to measure enzyme activity as well as the need to account for possible interference by hepatic supernatants. In brief, liver samples were homogenized (1:10, w/v) in 50 mM imidazole buffer (pH 7.4) containing 0.25 M sucrose and 10 mM β-mercaptoethanol. After centrifugation (30 min at 10,000 x *g*), the supernatant was incubated for 5 min at 37 C in 100 mM phosphate buffer (pH 7.0) containing 5 mM EDTA, 20 mM dithiothreitol and 500 nM [^{125}I]rT_3 (26.7 MBq/nmole; DuPont-New England Nuclear, Boston, MA). The assay mixture (0.1 ml) contained 30 to 45 μg protein. The released ^{125}I was isolated as TCA-soluble radioactivity and measured in a γ-scintillation counter. Results were identical with or without prior ion-exchange chromatography on columns of AG 50W-X8 (Bio-Rad Laboratories, Richmond, CA). Enzyme activity was expressed as nmoles of ^{125}I$^-$ produced per hour per mg protein. The assay was validated for chicken livers as suggested by McNabb *et al.* (14).

TABLE 1.

Composition Of The Experimental Diets (g/kg diet)

	Dietary energy as fat (MJ/kg diet)		
	1.25	2.5	5.0
Ingredient			
Isolated soy protein*	20	20	20
Soya-bean meal	250	250	250
Maize meal	450	450	450
Maize oil	14	47	114
Glucose	180	100	0
Sand	10	28	50
Dicalcium phosphate	40	40	40
Limestone	10	10	10
L-methionine†	5	5	5
Selenium premix‡‡	1	1	1
Mineral premix§	1	1	1
Vitamin premix‖	5	5	5
Cellulose	10	40	55
Calculated Composition			
metabolizable Energy (MJ/kg)	12.8	12.8	12.8

*Soya-bean protein grade II (900 g/kg crude protein, 21726); Nutritional Biochemicals, PO Box 22400, Cleveland, Ohio 44122, USA.
†L-methionine (18915), US Biochemicals, PO Box 22400, Cleveland, Ohio 44122, USA.
‡Provided 0.2 mg Se/kg of diet.
§Provided (mg/kg of diet): manganese 100, iron 100, copper 10, cobalt 1,iodine 1 and zinc 100.
‖Provided (mg/kg of diet): retinol 3.6, cholecalciferol 0.075, biotin 1, vitamin E 10, riboflavin 10, pantothenic acid 20, choline 2 g, niacin 100, thiamine 10, vitamin B_6 10, menadione sodium bisulfite 1.5, cyanocobalamin 0.1, folic acid 2 and ethoxyquin 150.

Product formation was proportional to incubation time and protein content (apparent K_m and V_{max} values for pooled control samples were 0.82 μM [^{125}I] rT_3 and 3.7 nmoles $^{125}I^- \cdot h^{-1} \cdot$ mg protein^{-1}, respectively). Protein concentration in homogenates was determined with bicinchoninic acid reagent (Pierce Chemical Co., Rockford, IL) and BSA as a standard (15).

Statistical Analysis

Data were analyzed by the General Linear Models (GLM) procedure of SAS (16). The statistical model included main effects of fat level, T_3 supplementation, and fat x T_3 as the interaction.

RESULTS AND DISCUSSION

Feeding diets containing T_3 (1 mg/kg diet) to growing chickens decreased feed intake ($P<0.01$) and body weight gain ($P<0.01$) regardless of fat content of the diet (Table 2). These data support our previous observations of the inhibitory effect of dietary T_3 (12) and T_4 (11) on growth of chickens.

TABLE 2.

Dietary energy and triiodothyronine (T_3) effects on broiler growth [Mean values and SE's for four observations per dietary treatment]

Fat energy (MJ/kg)	T_3 (mg/kg)	Body Wt (g)	Feed intake (g/bird for 7-28 d)	Gain/Feed intake (g/g)
1.25	0	1381 ± 41	2095 ± 17	0.56 ± 0.02
1.25	1	1015 ± 33	1559 ± 99	0.56 ± 0.03
2.5	0	1438 ± 30	2153 ± 83	0.57 ± 0.02
2.5	1	1044 ± 44	1547 ± 78	0.58 ± 0.02
5.0	0	1390 ± 45	2118 ± 92	0.56 ± 0.02
5.0	1	1100 ± 35	1657 ± 81	0.51 ± 0.04
P values				
Fat		<0.14	<0.77	<0.33
T_3		<0.01	<0.01	<0.58

Feeding diets with different amounts of energy from fat (1.25, 2.5, and 5.0 MJ/kg diet) did not affect plasma concentration of T_3 in chickens (Figure 1). On the other hand, T_3 supplementation increased ($p<0.001$) circulating T_3. Mean plasma T_3 concentration across dietary fat levels were 4.7 and 18.2 nmol/L (SEM = 0.44) for control and T_3 supplemented diets, respectively. No significant effect of dietary fat x T_3 interaction was found.

Likewise, plasma concentrations of T_4 (Figure 2) were not affected by different amounts of energy from dietary fat or by the dietary fat x T_3 interaction. In contrast, T_3 supplementation decreased ($P<0.001$) circulating T_4. Mean plasma T_4 concentrations, across

fat levels, were 12.4 and 7.9 nmol/L (SEM = 0.32) for control and T_3 supplemented diets, respectively. The changes in plasma T_4 concentrations in chickens after long term T_3 administration were relatively small in comparison to changes observed in mammals. In the present study, the average daily intake of 13 μg of T_3/100 g body weight (11) decreased plasma T_4 concentration 35.6%. In contrast, in growing steers, injections of 2 μg of T_3/kg BW on alternate days decreased plasma T_4 concentration 89.1% within 6 d (17). These data suggest that differences exist between birds and mammals in either the responsiveness of pituitary-thyroid axis to the negative feedback regulation by circulating T_3 or to species differences in thyroid hormone clearance rates.

Neither dietary fat nor dietary fat x T_3 interaction affected the molar ratio of plasma T_3: T_4. In contrast, supplementing diets with T_3 increased ($P<0.001$) the molar ratio. Mean plasma T_3: T_4 values, across fat levels, were 0.51 and 2.70 (SEM = 0.07) for control and T_3 supplemented diets, respectively.

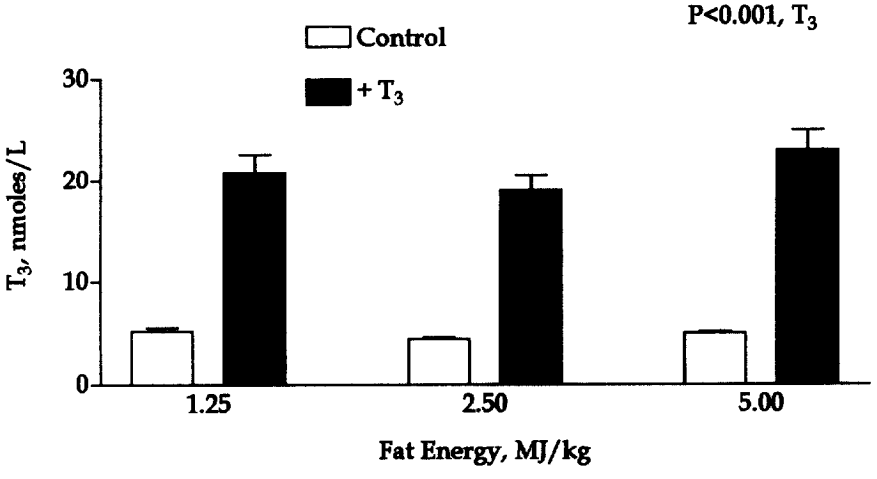

FIG. 1.

Effect Of Dietary Fat Content And Triiodothyronine (T_3) Supplementation On Plasma T_3.

The activity of hepatic 5'D (Figure 3) was affected by the dietary fat x T_3 interaction ($P<0.01$). Diets without T_3 but containing 2.5 and 5.0 MJ/kg from fat increased 5'D activity 25 ($P<0.01$) and 16% ($P<0.05$), respectively, as compared to diet containing 1.25 MJ from fat. In contrast, no changes in 5'D were observed when T_3 was included in the diets.

The data support previous observations in mammals that dietary fat stimulates hepatic 5'D activity (7,8). In the present experiment, increased dietary fat content enhanced 5'D activity in liver in spite of decreased concentrations of dietary glucose (Table 1), a known stimulator of extrathyroidal T_4 to T_3 conversion (6). Increased hepatic generation of T_3, which was not reflected in plasma concentration of T_3 and T_4 (Figures 1 and 2), indicates the importance of locally produced T_3 in the metabolic response to fat feeding.

Increased hepatic 5'D activity in chickens fed diets with high fat content may increase intracellular T_3 concentration as a compensatory measure to overcome the inhibitory effect of fatty acids on T_3 nuclear binding (18). Such an explanation is supported by the present data indicating that T_3 supplementation eliminates the stimulatory effect of fat on 5'D activity. Furthermore, in rats T_3 administration increases the activity of several lipogenic enzymes. This increase is attenuated by fat-supplemented diets (19). An alternate explanation for the increased hepatic 5'D activity is that intracellular T_3 concentration may stimulate the oxidation of exogenous fatty acids provided by dietary fats. Although T_3 stimulated fatty acid esterification requires *de novo* synthesized fatty acids, T_3 stimulated fatty acid oxidation requires exogenous fatty acids (20). The exact mechanism of the stimulatory effect of fat on extrathyroidal 5'D is not known at the present time, but preliminary data collected from steers suggested that certain fatty acids may directly stimulate hepatic 5'D activity *in vitro* (21). Later data suggested that various aspects of pituitary hormone regulation are differentially affected by FA composition. Fatty acid infusion may accentuate end organ responses in the thyroid axis and decrease IGF-I in the somatotropic axis. The data also suggest that fatty acid isomers may alter patterns of extrathyroidal generation of thyroid hormones via direct influences on 5'DIn contrast to mammalian data (4,22), T_3 administration did not stimulate hepatic 5'D activity in chickens regardless of fat content of the diet. In fact, T_3 feeding abolished the stimulatory effect on enzyme activity of feeding the diet containing 2.5 MJ from fat/kg diet

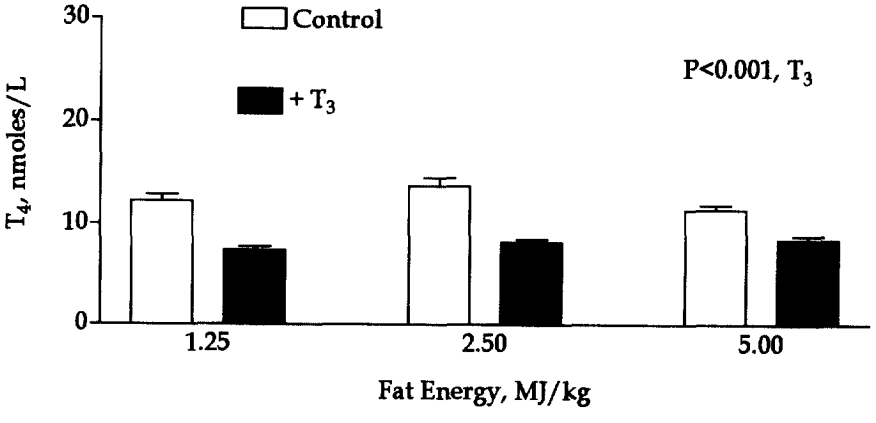

FIG. 2.

Effect Of Dietary Fat Content And Triiodothyronine (T_3) Supplementation On Plasma T_4.

In conclusion, it was shown that feeding growing chickens isocaloric and isonitrogenous diets containing increased amounts of calories from fat stimulated hepatic 5'-deiodinase activity without affecting circulating concentrations of T_3 and T_4. These findings indicate that extrathyroidal T_3 generation and, consequently, intracellular T_3 concentration may be affected

by dietary fat. In addition, feeding diets supplemented with T_3 increased the molar ratio of T_3 to T_4 in plasma, attenuating the effect of dietary fat on hepatic activity of 5'-deiodinase.

The results of the present study strongly suggest that hepatic T_4 to T_3 conversion may be involved in the regulation of circulating T_3 concentrations during the feeding of high fat diets. In addition, the data from this study also show that T_3 regulates the availability of its precursor, T_4. It should be noted, however, levels of fat used in the diets in the present study are not typical of those encountered in normal broiler diets.

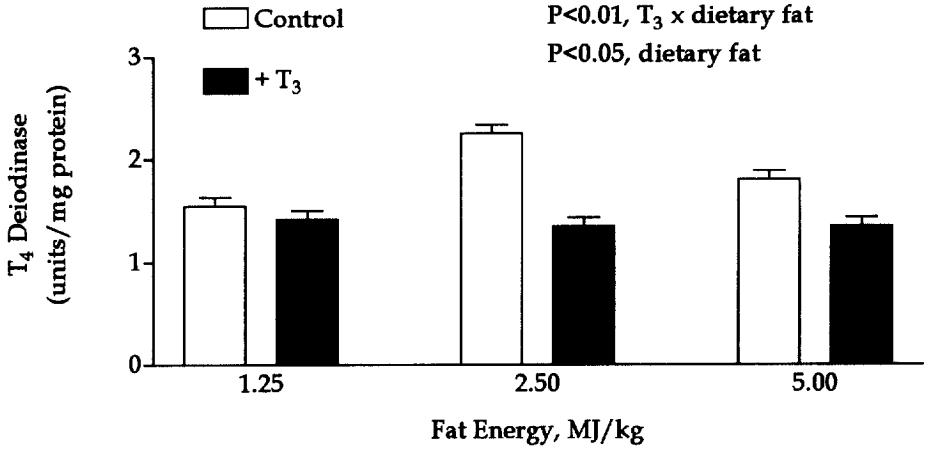

FIG. 3.

Effect Of Dietary Fat Content And Triiodothyronine (T_3) Supplementation On Hepatic 5'-Deiodinase (Type I 5'D) Activity.

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